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The antiquity of microbial sulfate reduction

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Abstract

The phylogenetic positions of sulfate-reducing organisms, as revealed from comparisons of small-subunit ribosomal RNA (SSU rRNA), are spread over both the Archaeal and Bacterial domains, though when they evolved is uncertain. The low-branching positions of some of these groups on the Tree of Life have inspired the hypothesis that the metabolic innovation of microbial sulfate reduction is of great antiquity. Only recently, however, have sulfur isotope data from Precambrian rocks begun to emerge that clearly demonstrate sulfate-reducing microbes had evolved by the early Archean. The large spread of $\delta^{34}S$ values of microscopic pyrites aligned along growth faces of former gypsum crystals in the ~3.47-Ga North Pole barite deposit of northwestern Australia provide the oldest evidence of microbial sulfate reduction and the earliest indication of a specific microbial metabolism. The distinct expression of microbial sulfate reduction in this localized and cool sulfate-rich environment provides the oldest date for calibrating the temporal progress of early evolution on the Tree of Life.

Keywords: Archean; Gypsum; Barite; Pyrite; Sulfur isotopes; Sulfate reduction; Microbial metabolism; Early evolution

1. Introduction

In 1886, a German scientist, F. Hoppe-Seyler, published a seminal paper in "Zeitschrift für Physiologie und Chemie". According to this report, when gypsum ($CaSO_4 \cdot 2H_2O$) was added to anaerobic mud enrichments containing organic matter, cellulose was completely oxidized and "rotten egg" gas (H_2S) was produced (Hoppe-Seyler, 1886). However, at that time, just how sulfate was transformed into stinking gas was quite unclear. But today, we know that microorganisms are to blame and call this process "microbial sulfate reduction". It is important for the maintenance of the sulfur biogeochemical cycle and thus influences the abundance of pyrite in sedimentary rocks, the survival or organic matter in sedimentary basins and ultimately the redox state of the Earth's hydrosphere, atmosphere and lithosphere. Without it, some styles of metal deposits would not form and diagenetic mineral assemblages would be markedly different. Truly, it is a vital component of the Earth system as we know it.

Microbial sulfate reduction is an energy-yielding metabolic process during which sulfate is reduced and sulfide is released, coupled with the oxidation of organic matter or hydrogen (H_2) (Postgate, 1984).

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Sulfate-reducing microbes are obligate anaerobes that are widely distributed in anaerobic marine and terrestrial environments (e.g., Widdel, 1988), though they may survive temporary exposure to oxygen and again become active under anaerobic conditions (Canfield and Des Marais, 1991; Labrenz et al., 2000; Cypionka, 2000; and references therein). As a group, sulfate-reducers can thrive under a wide range of ecological conditions from extremely cold habitats (e.g., Sagemann et al., 1998) to active hydrothermal vents (Jørgensen et al., 1992). Sulfate-reducers are also phylogenetically diverse (Widdel, 1988; Devereux and Stahl, 1993; Castro et al., 2000), with some groups occupying deeply rooted branches on the Tree of Life (Stackebrandt et al., 1995; Pace, 1997; Wagner et al., 1998). As such, the group may contain some of the oldest life forms on Earth. Thus, understanding the evolution and metabolism of sulfate-reducers is of great scientific interest, because they may represent something like the primordial state of life.

In order to investigate the early history of this metabolic system, there are three possible lines of approach, given current knowledge and technology. Firstly, one could search for the preserved remains of the organisms themselves, the ideal form of evidence (Buick, 2001). This approach has proved very successful for researching the antiquity of other metabolic systems such as oxygenic photosynthesis, where some relevant microbes have distinctive cellular structures (Schopf, 1983; Schopf and Klein, 1992; Knoll, 1996). However, it does not work very well for sulfate-reducers. Although they are phylogenetically very diverse, as mentioned above, they are morphologically simple, either unadorned spheroids or basic filaments, and thus may be difficult to distinguish from most other microbial fossils (Knoll, 1992). One might assume that a better strategy would be to search for microfossils encrusted or replaced by the products of sulfate reduction, i.e., pyrite. However, secondary pyritic replacement of organic matter is quite common in the geologic record and it is often difficult to discriminate from primary mineralization, especially in small objects. For example, thread-like pyritic filaments in a 3.2-Ga deep-sea volcanogenic massive sulfide deposit probably represent thermophilic prokaryotes, implying that life existed in Archean submarine hydrothermal spring systems (Rasmussen, 2000). But it is unclear whether the microbes were sulfate-reducers, because they occur in a setting where vigorous secondary sulfide mineralization took place as a result of inorganic hydrothermal precipitation. So searching for microfossils of sulfate-reducers is fraught with difficulty and, indeed, no certain cellular remains of such microbes have ever been identified.

The second method is to look for molecular fossils (commonly called biomarkers), distinctive organic compounds produced by a particular group of organisms that are preserved in modified but recognizable form in ancient sedimentary rocks (Summons and Walter, 1990). For instance, diverse cholesterols are ubiquitous in eukaryotes but are almost never synthesized by prokaryotes, so the extraction of derivative C_{27}–C_{29} cholestanes from 2700 Ma old organic-rich black shales provides convincing evidence for the early evolution of our lineage (Brocks et al., 1999). Some biomarkers are so specific that they can provide evidence for the existence of a particular metabolism. 2α-Methylhopanes with 31 carbon atoms or more, which are geolipids derived from hopanol biolipids with a similar carbon skeleton, are diagnostic in high concentrations of cyanobacteria and hence are indicative of oxygenic photosynthesis (Summons et al., 1999). Unfortunately, though some sulfate-reducers produce recognizable biomarker molecules such as 10Me16:0 and i17:1 ester-bound fatty acids (Taylor and Parkes, 1985), none of these survive rigorous geological conditions in recognizable form and are thus only found in young sediments. Hence, at present, biomarker studies shed no light on the early evolution of this metabolic process.

So, we are left with isotopes. Isotopic measurements of biological elements such as carbon and nitrogen have been widely used to study early evolution, because the isotopic fractionations of these elements imparted by biological and non-biological processes usually have distinct signatures (e.g., Schidlowski, 1988; Beaumont and Robert, 1999). The same is true for sulfur. During microbial sulfate reduction, the stable isotopes ^{32}S and ^{34}S are discriminated so that the daughter sulfides are isotopically fractionated with respect to the parent sulfate, with the sulfides being depleted in ^{34}S (e.g., Thode et al., 1951; Chambers and Trudinger, 1979). Though

inorganic processes can also produce sulfide minerals with similarly large depletions in ^{34}S, they do not operate under low-temperature conditions. Therefore, the S-isotope signature of sedimentary sulfides can be a powerful tool for detecting signs of sulfate-reducing prokaryotes. As this signal is not substantially altered under moderate post-depositional regimes, the potential thus exists for tracing the isotopic signature of microbial sulfate reduction back through time for as long as well-preserved rocks survive. Indeed, a great many sulfur isotopic measurements of Precambrian sedimentary rocks have clarified our understanding of the more recent evolution of sulfate-reducing microorganisms (Goodwin et al., 1976; Lambert et al., 1978; Monster et al., 1979; Cameron, 1982; Strauss, 1986; Ohmoto et al., 1993; Strauss and Beukes, 1996; Kakegawa et al., 1999; Grassineau et al., 2000; England et al., 2002). But we still know little about the operation of the sulfur cycle during the first third of Earth's history—the earlier part of the Archean eon, that time before 3.0 billion years ago.

The S-isotopic geochemistry of Archean rocks and the evolution of the early Precambrian sulfur cycle have previously been reviewed in considerable detail by many workers (Schidlowski et al., 1983; Lambert and Donnelly, 1990; Hayes et al., 1992; Ohmoto, 1992; Knoll and Canfield, 1998; Canfield, 2001a; Canfield and Raiswell, 1999; Strauss, 1999, 2002). In this paper, we will highlight some advances in our understanding of metabolic processes and isotopic fractionation during sulfate reduction. In light of our recent study of ~ 3.47-Ga-old barite and pyrite from North Pole in Australia (Shen et al., 2001), we will then focus on the early Archean isotopic record and discuss how these advances constrain the early evolution of microbial sulfate reduction and its implications for primordial ecosystems.

2. Measurement of sulfur isotopes and fractionations

Differences in sulfur isotopic composition are commonly expressed in terms of the conventional δ-notation giving the parts per thousand or per-mil (‰) deviation of the isotope ratio of a sample relative to the standard Cañon Diablo troilite (CDT) that defines zero per mil on the δ-scale, i.e.

$$\delta^A S \text{ in } ‰ = [(^A S/^{32} S)_{sample}/(^A S/^{32} S)_{st} - 1] \times 1000$$

where $A = 34$, 33 or 36. CDT has been a convenient standard because it represents meteoritic sulfur which is very close to the bulk Earth value. Recently, however, a new V-CDT scale defined by the reference material IAEA-S-1 (Ag_2S) has been proposed (Ding et al., 2001) because CDT is no longer available in large quantities and is also slightly inhomogeneous in its isotopic composition (e.g., Beaudoin et al., 1994).

Most study to date has been of $\delta^{34}S$, because the two isotopes involved are the most abundant in nature: ^{32}S (95.02%) and ^{34}S (4.21%), and therefore $\delta^{34}S$ measurements are quite straightforward. ^{33}S and ^{36}S have received less attention because their natural abundances are much lower (0.75% and 0.02%, respectively) and thus isotopic measurements, particularly of ^{36}S, are difficult and time-consuming. It was also assumed that both biological and chemical processes generally fractionate isotopes according to their relative mass differences (i.e., mass-dependent) such that $\delta^{33}S$ and $\delta^{36}S$ are quantitatively correlated with $\delta^{34}S$, providing little new information. The mass-dependent S-isotopic fractionations follow the approximate linear relationships $\delta^{33}S = 0.515 \times \delta^{34}S$ and $\delta^{36}S = 1.91 \times \delta^{34}S$ (Hulston and Thode, 1965). However, after examining the $\delta^{33}S$, $\delta^{34}S$ and $\delta^{36}S$ records of Archean rocks, Farquhar et al. (2000) determined that $\delta^{33}S$ and $\delta^{36}S$ did not necessarily follow mass-dependent arrays during early Earth history. These anomalous signatures have been termed mass-independent fractionations, which are quantitatively defined as $\Delta^{33}S \neq 0$ where $\Delta^{33}S = \delta^{33}S - 0.515 \times \delta^{34}S$ and likewise for $\Delta^{36}S$ (Farquhar et al., 2000).

3. Microbial sulfate reduction and isotopic fractionations

Quantitatively, sulfur constitutes on average about 1% of the dry mass of living things, residing principally in the amino acids cysteine and methionine. The latter constitutes the starting component of all proteins, the chief catalytic and structural molecules in cells, and

so sulfur is essential for life as we know it. Cellular sulfur is mainly in its most reduced state (-2), but most accessible sulfur in the ambient environment is in an oxidized state. Therefore, sulfate must be reduced to H_2S in order to be biochemically useful. All green plants, fungi, and most bacteria can reduce sulfate to sulfide (H_2S) that is subsequently incorporated into sulfur-containing organic molecules (Roy and Trudinger, 1970). This metabolic pathway is known as assimilatory reduction, which is an energy-requiring process. By contrast, dissimilatory sulfate reduction is an energy-yielding reaction that is carried out by several groups of prokaryotes that use it to obtain energy for growth and maintenance (Postgate, 1984).

3.1. Assimilatory sulfate reduction

3.1.1. Biochemical process

Since all reductive steps from sulfate to sulfide occur in the cytoplasm, sulfate must be transported across the cell membrane. In terms of kinetics, sulfate seems to be a rather sluggish ion. Also, the free sulfate dianion is not itself a suitable electron acceptor. Thus, the first step in assimilatory sulfate reduction is to activate external sulfate (Peck and Lissolo, 1988) (Fig. 1, step 1). The uptake of sulfate is unidirectional (i.e., no exchange between external and internal sulfate) and requires ATP that is only available within the cytoplasm (Eq. (1)):

$$SO_4^{2-} + ATP \leftrightarrow APS + PPi \quad (1)$$

where APS = adenosine phosphosulfate and PPi = pyrophosphate.

This reaction catalyzed by ATP sulfurylase is endergonic and pulled by the subsequent hydrolysis of pyrophosphate by pyrophosphatase (Eq. (2)), thus favoring APS formation (e.g., Fauque et al., 1991; Fig. 1, step 2) (Eq. (3)):

$$PPi + H_2O \rightarrow 2Pi \quad (2)$$

where Pi = orthophosphate.

Sum reactions (1) and (2):

$$SO_4^{2-} + ATP + H_2O \rightarrow APS + 2Pi \quad (3)$$

Following the formation of APS, there are two possible pathways involved: the APS assimilatory pathway and the PAPS assimilatory pathway. For the latter, an energy-rich phosphoryl group is spent in a reaction catalyzed by APS kinase, yielding 3′-phosphoadenosine 5″ phosphosulfate (PAPS) and ADP (e.g., Fuchs, 1999) (Fig. 1, step 3) (Eq. (4)):

$$APS + ATP \rightarrow PAPS + ADP \quad (4)$$

Then, PAPS is reduced to sulfite by PAPS reductase (Eq. (5)). Subsequently, sulfite is reduced to sulfide by sulfite reductase (Fig. 1, steps 4–5) (Eq. (6)):

$$PAPS + [H] \rightarrow 3'\text{-phospho-AMP} + SO_3^{2-} \quad (5)$$

$$SO_3^{2-} + 6[H] \rightarrow HS^- + 3H_2O \quad (6)$$

where AMP = adenosine monophosphate and [H] represents the electron donor.

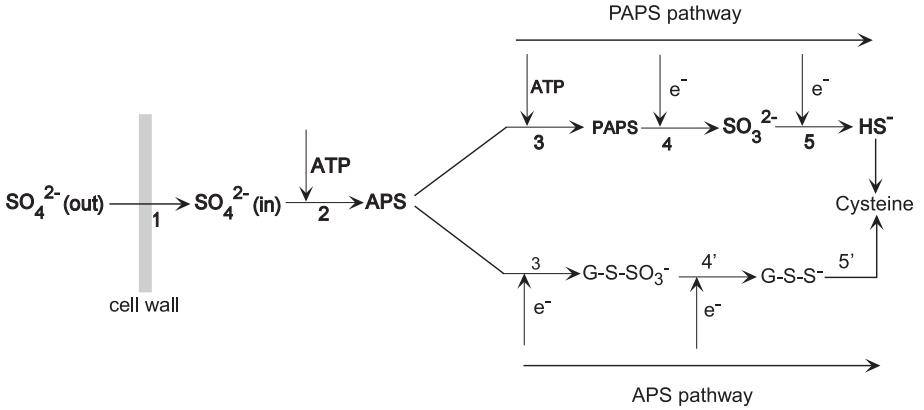


Fig. 1. The biochemical pathways of assimilatory sulfate reduction.

The resulting sulfide is immediately trapped in cysteine by *O*-acetylserine sulfhydrylase, from which other sulfur-containing compounds ultimately obtain their sulfur (Fig. 1). The APS assimilatory pathway differs from the PAPS pathway in that the first reduction involves APS with the formation of a thiosulfonate ion ($-S-SO_3^-$) (Fig. 1, step 3′) rather than sulfite, which is in turn reduced to form cysteine (Fig. 1, steps 4′–5′).

In both pathways, assimilatory sulfate reduction creates a cycle between inorganic and organic states of sulfur and does not produce significant amounts of free H_2S (Siegel, 1975). There is evidence, however, that these metabolic pathways may be modified by abnormal nutritional factors and in some circumstances release traces of free sulfide (Krouse et al., 1984).

3.1.2. Isotopic fractionation

The overall S-isotopic fractionation during assimilatory sulfate reduction is small. Aquatic plants and animals from both fresh-water and marine environments generally display little isotopic discrimination (+0.5‰ to −4.4‰) in organic sulfur relative to external sulfate (Kaplan et al., 1963; Mekhtieva and Pankina, 1968). Only small fractionations (−0.9‰ to −2.7‰) between cellular sulfur and initial sulfate have been reported during growth of microorganisms (Kaplan and Rittenberg, 1964; Chambers and Trudinger, 1979). A recent summary shows that the average fractionation between $\delta^{34}S$ of plants and their sulfate source is about −1.5‰ (Trust and Fry, 1992), thus reinforcing the conclusion that assimilatory sulfate reduction only results in slight isotopic fractionation.

It is generally accepted that the isotopic effects during the uptake of sulfate (Fig. 1, step 1) are small, usually less than about 3‰ (Harrison and Thode, 1958; Rees, 1973). But the subsequent reduction of sulfite to sulfide can produce a large fractionation of up to 41‰ (Kaplan and Rittenberg, 1964). Thus, it is unclear that why the large fractionation produced by sulfite reduction is not reflected in the overall isotopic fractionation during assimilatory sulfate reduction. It has been suggested that as the reaction proceeds unidirectionally without exchange between external and internal sulfate pools (Fig. 1), the overall isotopic effect is necessarily limited to that in the first step, the uptake of sulfate (Rees, 1973). As such, the overall isotopic fractionation is small even if sulfite reduction imparts large fractionations to part of the intracellular sulfur pool, because the residuum will be constrained in the closed system to show a complementary opposite fractionation.

3.2. Dissimilatory sulfate reduction and isotopic fractionation

Sulfate-reducing Bacteria and Archaea are able to use sulfate ions as electron acceptors for anaerobic respiration (Widdel, 1988; Stetter, 1996). This metabolic pathway is named dissimilatory sulfate reduction. As far as is known, it is performed by members of five phylogenetically distinct but morphologically similar groups of microbes. The only genus within the Archaea with this metabolism is the hyperthermophilic (growth at >70 °C) euryarchaeote *Archaeoglobus*, which occupies a fairly peripheral phylogenetic branch alongside the halophiles and some methanogens. The deepest-branching Bacterial group of sulfate-reducers is also hyperthermophilic, consisting only of the genus *Thermodesulfobacterium*. The medially branching Nitrospirae phylum contains the thermophilic (growth at 40–70 °C) sulfate-reducing genus *Thermodesulfovibrio*. The peripheral group Firmicutes (also called the *Bacillus*/*Clostridium* group or the low G+C gram-positive bacteria) contains the genera *Desulfotomaculum* and *Desulfosporosinus*. By far the greatest diversity of sulfate-reducers resides within the δ-subdivision of the Proteobacteria, including the thermophilic genus *Thermodesulforhabdus* and over a dozen mesophilic (growth between about 15 and 45 °C) genera including the archetypal sulfate-reducer *Desulfovibrio*. Several other microbes possess parts of the sulfate-reduction metabolic pathway, but they seem to use it for other purposes such as sulfide oxidation or sulfite respiration. Clearly, the metabolism is widespread among the prokaryotes and has been hypothesized as ancestral (Wagner et al., 1998). However, more recent genetic studies suggest that it has been transferred between lineages several times during ancient and more recent evolutionary history (Klein et al., 2001).

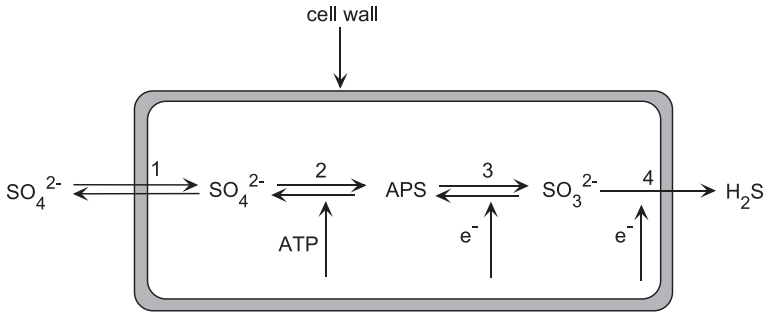


Fig. 2. The biochemical pathway of dissimilatory sulfate reduction.

3.2.1. Biochemical process

Dissimilatory microbial sulfur transformations are closely linked to the carbon cycle because sulfate reduction is usually coupled with organic oxidation (Eq. (7)). Molecular hydrogen (H_2) may also serve as electron donor (Eq. (8)):

$$SO_4^{2-} + CH_2O \rightarrow H_2S + HCO_3^- \quad (7)$$

$$SO_4^{2-} + H_2 \rightarrow H_2S + H_2O \quad (8)$$

The biochemical processes of dissimilatory sulfate reduction can be divided into two steps: endergonic reduction to sulfite catalyzed by soluble enzymes (steps 1–3), followed by exergonic sulfite reduction to sulfide catalyzed by membrane-bound enzymes (step 4) (Fig. 2). However, the actual biochemical pathway may be more complicated and variable between microbial species (Widdel and Hansen, 1992). Assimilatory and dissimilatory reductions are similar in that ATP sulfurylase is common to both and results in the formation of APS that is further reduced to sulfite by APS reductase (Eqs. (1)–(3)). However, in contrast to assimilatory sulfate reduction, the transition from external sulfate to sulfite in dissimilatory sulfate reduction is reversible (Fig. 2). This reversible system may allow kinetic isotope effects to be expressed during sulfite reduction (Rees, 1973).

Two pathways of sulfite reduction to sulfide have been proposed: via trithionate and by direct reduction of sulfite to sulfide (Widdel and Hansen, 1992; Cypionka, 1995) (Fig. 3). The first step in the trithionate pathway (Kobayashi et al., 1969; Kim and Akagi, 1985) is the reduction of sulfite to trithionate ($^-O_3S\text{-}S\text{-}SO_3^-$) by sulfite reductase (Eq. (9)) (Fig. 3, step 1):

$$3HSO_3^- + 2e^- + 3H^+ \rightarrow {}^-O_3S\text{-}S\text{-}SO_3^- + 3H_2O \quad (9)$$

A further two-electron reduction by trithionate reductase yields thiosulfate ($^-S\text{-}SO_3^-$) that is finally

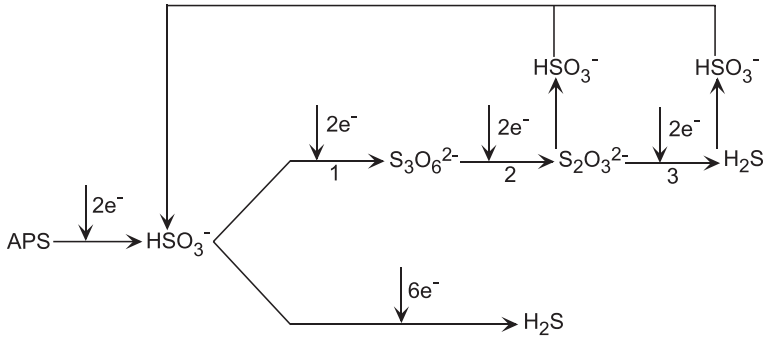


Fig. 3. The proposed pathways of sulfite reduction (simplified from Widdel and Hansen, 1992).

reduced to sulfide by thiosulfate reductase (Eqs. (10)–(12)) (Fig. 3, steps 2–3):

$$^{-}O_3S\text{-}S\text{-}SO_3^- + 2e^- + H^+ \rightarrow {}^{-}S\text{-}SO_3^- + HSO_3^- \quad (10)$$

$$^{-}S\text{-}SO_3^- + 2e^- + 3H^+ \rightarrow HSO_3^- + H_2S \quad (11)$$

Sum Eqs. (10) and (11):

$$HSO_3^- + 6e^- + 7H^+ \rightarrow H_2S + 3H_2O \quad (12)$$

It has been reported that neither trithionate nor thiosulfate are obligatory intermediates in this pathway, forming instead by chemical side reactions in the culture medium as a result of high sulfite concentrations (Chambers and Trudinger, 1975; Trudinger and Loughlin, 1981). However, recent studies of whole cells show the formation of trithionate and thiosulfate in growing cultures and in washed cells (Fitz and Cypionka, 1990). Hence, sulfite reduction by the trithionate pathway may be a fully functional biochemical process.

The direct reduction of sulfite to sulfide involves six-electron reduction catalyzed by sulfite reductase and yields sulfide through a single step only (Fig. 3). In both the trithionate and the direct reduction pathways, dissimilatory sulfate reduction releases large amounts of free sulfides as the sole final product (e.g., Widdel, 1988). The turnover rates of sulfur in dissimilatory processes exceed those during assimilatory reduction by several orders of magnitude.

3.2.2. Isotopic fractionation

Though the uptake of sulfate into the cell and then by ATP sulfurylase are associated with only small fractionations (Rees, 1973), reductive processes in the cytoplasm are accompanied by large fractionations, because APS reduction to sulfite and sulfite reduction to sulfide involve the breaking of S−O bonds. During these processes, the lighter sulfur (^{32}S) tends to react faster than heavier sulfur (^{34}S) because the bonding energy of ^{32}S–O is smaller and thus breaking this bond is easier than for ^{34}S–O. Hence, the product H_2S is enriched in ^{32}S and depleted in ^{34}S.

In a pioneering study, Thode et al. (1951) reported that in open systems *Desulfovibrio desulfuricans* produced sulfide enriched by approximately 10‰ in ^{32}S relative to source sulfate. Continuing studies of isotopic fractionation during sulfate reduction by pure microbial cultures have shown large fractionations ranging from 2‰ to 46‰ (Harrison and Thode, 1958; Kaplan and Rittenberg, 1964; Kemp and Thode, 1968; Chambers et al., 1975). The considerable variations in isotopic fractionations may be influenced by many factors including sulfate concentration, electron donor, temperature, specific rate of sulfate reduction and growth conditions, according to Chambers and Trudinger (1979). These variables will be examined in more detail below.

In general, the earlier work on *Desulfovibrio desulfuricans* showed that the highest fractionations were obtained at low rates of sulfate reduction, whereas the minimum isotope effect at high reduction rates approached 0‰. Thus, the degree of isotopic fractionation seemed to be an inverse function of specific sulfate reduction rate (rate per microbial cell) (Harrison and Thode, 1958; Kaplan and Rittenberg, 1964; Chambers et al., 1975). As a result, the influence of temperature and electron donors on isotopic fractionation has been interpreted in terms of their effects on cell-specific sulfate reduction rates (Harrison and Thode, 1958; Kaplan and Rittenberg, 1964; Kemp and Thode, 1968; Chambers et al., 1975). But it has been also noted, for instance by Chambers and Trudinger (1979), that there is a wide variation in fractionation at any given rate of reduction. More recent pure culture studies reinforce the earlier results with sulfide formed during sulfate reduction depleted in the isotope ^{34}S by 4–49‰ (Bolliger et al., 2001). Under optimized conditions with respect to electron donors, salinity, temperature and pH, batch cultures performed with 32 different sulfate-reducing microbes representing 28 different genera also show a similar isotopic fractionation range from 2‰ to 42‰ (Detmers et al., 2001) (Fig. 4). Significantly, sulfate-reducers that oxidized the carbon source completely to CO_2 show greater fractionation than those that release acetate as the final product of carbon oxidation during sulfate reduction (Detmers et al., 2001).

While pure culture studies are valuable for understanding sulfate reduction under controlled laboratory conditions, things are different in nature. For example, microbes in nature do not form resting cell suspensions; instead they continually multiply and die. In pure cultures, organic substrate is normally

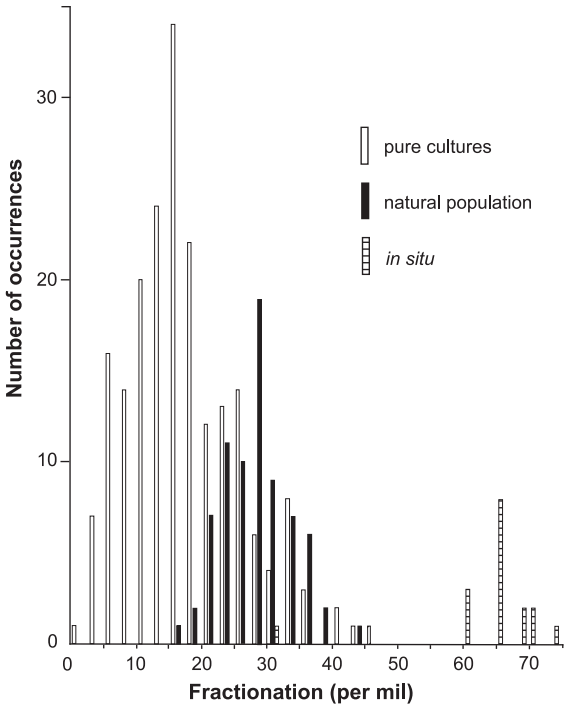


Fig. 4. The histogram of sulfur isotope fractionation by pure cultures and natural populations. The data from Habicht and Canfield (1997), Detmers et al. (2001), Brüchert et al. (2001) and Wortmann et al. (2001).

supplied in excess, but in natural environments organic substrate may be limited (e.g., Morita, 1987), as in chemostat experiments (e.g., Chambers et al., 1975). In addition, in batch culture experiments microbes commonly grow significantly faster than in natural environments. Thus, studies of natural populations of sulfate-reducing microbes are required to properly understand isotopic fractionation in the wild state.

Recent work has shown that natural populations of sulfate-reducing microbes in surface environments produce comparable fractionations to pure cultures, with an upper limit of 45‰ (Habicht and Canfield, 1997; Canfield, 2001b). Minimum fractionations are never lower than 10‰ in sediments where abundant sulfate is available (Habicht and Canfield, 1996, 2001), unless excess organic substrate is added (Böttcher et al., 1997). Though reduced fractionations of 3–19‰ are observed when H_2 serves as the electron donor (Kaplan and Rittenberg, 1964; Kemp and Thode, 1968), conditions where hydrogen is more abundant than organic matter are rarely encountered in natural environments. So, it is likely that in natural environments at sulfate concentrations greater than 1 mM, sulfides are isotopically fractionated by 10–40‰ compared to the sulfate (Canfield and Raiswell, 1999). It should be noted, however, that S-isotopic fractionation of around 30‰ has also been observed in culture experiments with toluene at sulfate concentrations as low as 300 μM (Bolliger et al., 2001). But small fractionations of <6‰ are invariably reported when sulfate concentrations are less than 200 μM (Habicht et al., 2002).

As discussed above, previous batch-culture, continuous-culture and resting-cell experiments suggested that for *Desulfovibrio* spp. the degree of isotopic fractionation was an inverse function of the rate of sulfate reduction (Kaplan and Rittenberg, 1964; Kemp and Thode, 1968; Chambers et al., 1975). However, recent data on isotopic fractionation by both pure cultures and natural populations of sulfate-reducing microbes show that things are more complicated. Isotopic fractionations during sulfate reduction by sulfate-reducers from psychrophiles to thermophiles have manifestly proved to be independent of specific reduction rates (Fig. 5). Though the reasons for the differences between the new data and the previous results are not clear, the sulfate-reducers in early experiments may have experienced physiological stress due to unfavorable growth temperatures or substrates, thus producing unrepresentative isotopic signatures (Brüchert et al., 2001).

According to early reports, the influence of temperature on isotope fractionation was generally explained by its effect on the rate of reduction (Kaplan and Rittenberg, 1964; Kemp and Thode, 1968). However, a wider survey of sulfate-reducers adapted to particular temperature régimes reveals otherwise. A compilation of data for various sulfate-reducers shows little relationship between natural sulfate reduction rates and temperature (Canfield et al., 2000). In terms of isotopic fractionation itself, pure culture studies using lactate as the electron donor with the mesophilic *Desulfovibrio desulfuricans* show that temperature has little influence on isotopic fractionation over a wide temperature range (Böttcher et al., 1999). These observations have been confirmed by studies of sulfate-reducers adapted to different temperature régimes. Sulfur isotope fractionations performed by cold-adapted (psychrophilic)

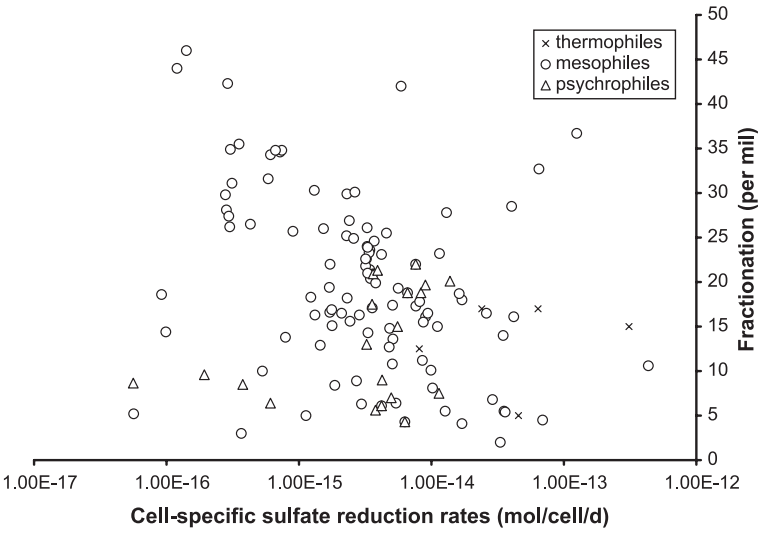


Fig. 5. The relationship between sulfur isotope fractionation and sulfate reduction rates. Data source: Psychrophiles: Brüchert et al. (2001) and Detmers et al. (2001); Mesophiles: Kaplan and Rittenberg (1964), Chambers et al. (1975) and Detmers et al. (2001); Thermophiles: Böttcher et al. (1999) and Detmers et al. (2001).

sulfate-reducers from Arctic marine sediments vary by less than 5.8 ‰ over a wide range of temperatures and rates (Brüchert et al., 2001). At the other extreme, pure cultures and natural populations of thermophilic sulfate-reducers metabolizing at temperatures between 50 and 85 °C yield fractionations of 13–28 ‰, with no linear correlation between temperature, reduction rate and isotopic fractionation (Böttcher et al., 1999; Canfield et al., 2000; Detmers et al., 2001). So, in contrast to earlier studies, wider exploration over a broad taxonomic range using different electron donors at different temperatures in pure cultures or in natural populations demonstrates that temperature and sulfate reduction rates have no systematic control on S-isotopic fractionations.

In sum, several general conclusions can be reached. First, with abundant sulfate at concentrations of more than 1 mM, pure cultures and natural populations of sulfate-reducers produce comparable fractionations with upper limits of 49 ‰. Second, at sulfate concentrations above 1 mM and possibly above 200 μM, there appears to be a minimum fractionation of approximately 9–10 ‰ in natural populations and pure cultures that use organic electron donors (Chambers and Trudinger, 1979; Habicht and Canfield, 1997), though this may differ for species using hydrogen as an electron donor. Third, sulfate-reducers that completely oxidize their carbon source fractionate more significantly than incompletely oxidizing cultures, possibly caused by the free energy difference between the two metabolic pathways (Detmers et al., 2001; Brüchert et al., 2001). Fourth, there is no inverse correlation between isotopic fractionation and cell-specific reduction rates, and temperature has no systematic effect on isotopic fractionation (Habicht and Canfield, 1996, 1997, 2001; Canfield et al., 2000; Detmers et al., 2001; Brüchert et al., 2001; Bolliger et al., 2001).

There are reasons to be cautious about these conclusions, however. Although over 100 species of sulfate-reducing microorganisms are known (e.g., Widdel, 1988; Castro et al., 2000), most of our knowledge on isotope fractionations comes from only a small proportion of them. It is quite possible that a wider survey might change our perception of biochemical processes and isotopic fractionations during sulfate reduction. As detailed ecological studies of natural sulfate-reducing communities show that there is species partitioning between differing physical habitats (Llobet-Brossa et al., 1998; Li et al., 1999; Ravenschlag et al., 2000), unusual environments may host unknown sulfate-reducers with abnormal physiological states that could influence isotopic fractionations. Indeed, new results suggest that sulfate-reducing com-

munities and their cellular metabolic activities in the deep biosphere may be different from those in near-surface sediments or the water column (Wortmann et al., 2001; Rudnicki et al., 2001). Coexisting dissolved sulfide and sulfate from hypersulfidic interstitial waters in deep subsurface ocean sediments as well as modeling of pore-water sulfate profiles in deep marine sediments show unusual fractionations up to 85‰ resulting from in situ dissimilatory sulfate reduction (Wortmann et al., 2001; Rudnicki et al., 2001) (Fig. 4). Hence, sulfate reduction in poorly known habitats may produce differing isotopic fractionations to those from well-studied settings and so may have important implications for our interpretation of S-isotope records in ancient sedimentary rocks.

4. H_2S oxidation and S-isotopic disproportionation

Another important issue for interpreting S-isotopic records over geological time is the re-oxidation of H_2S produced by sulfate reduction and its accompanying disproportionation. In modern marine environments, up to 90% of H_2S can be re-oxidized (Jørgensen, 1982), involving the formation of sulfur compounds of intermediate redox state such as elemental sulfur (S^0), sulfite (SO_3^{2-}), and thiosulfate ($S_2O_3^{2-}$) (e.g., Jørgensen, 1988; Zhang and Millero, 1993; Zopfi et al., 2001). These intermediate sulfur compounds do not accumulate under natural conditions because they are readily transformed by microbial oxidation, reduction or disproportionation. Disproportionation into sulfate and sulfide requires neither an electron donor nor an electron acceptor (Bak and Cypionka, 1987) and may follow differing pathways depending on which sulfur species (SO_3^{2-}, $S_2O_3^{2-}$, S^0) is involved (Cypionka, 1994), as shown below:

$$4SO_3^{2-} + H^+ \rightarrow HS^- + 3SO_4^{2-} \quad (\Delta G^o = -236\ \mathrm{KJ/mol}) \tag{13}$$

$$S_2O_3^{2-} + H_2O \rightarrow HS^- + SO_4^{2-} + H^+ \quad (\Delta G^o = -25\ \mathrm{KJ/mol}) \tag{14}$$

$$4S^0 + 4H_2O \rightarrow 3HS^- + SO_4^{2-} + 5H^+ \quad (\Delta G^o = +33\ \mathrm{KJ/mol}) \tag{15}$$

These reactions (Eqs. (13) and (14)) demonstrate that, under standard conditions, the coexistence of the most reduced (-2) and the most oxidized state ($+6$) of sulfur is energetically more stable than any of its intermediate oxidation states. They also indicate that the disproportionation of S^0 is an endergonic process (Eq. (15)) and therefore requires efficient removal of the evolved sulfide in order to make the reaction exergonic. In this regard, Fe oxyhydroxides can play an important role in buffering sulfide to low concentrations and can thus drive the S^0 disproportionation process (Thamdrup et al., 1993):

$$3HS^- + 2FeOOH + 3H^+ \rightarrow S^0 + 2FeS + 4H_2O \quad (\Delta G^{o'} = -144\ \mathrm{KJ/mol\ S^0}) \tag{16}$$

Sum Eqs. (15) and (16):

$$3S^0 + 2FeOOH \rightarrow SO_4^{2-} + 2FeS + 2H^+ \quad (\Delta G^{o'} = -34\ \mathrm{KJ/mol\ S^0}) \tag{17}$$

It has been demonstrated that microbial disproportionation of sulfur compounds of intermediate redox state can generate significant isotopic fractionations (e.g., Habicht et al., 1998). Disproportionation of elemental sulfur by pure and enrichment cultures produces sulfide that is depleted in ^{34}S by a moderate 5.5–8.6‰ (Canfield and Thamdrup, 1994; Böttcher et al., 2001). Through repeated cycles of H_2S oxidation and disproportionation of sulfur intermediate compounds, the total isotopic difference between sulfate and sulfide could significantly increase, though the quantitative impact on isotopic discrimination remains to be proven. Recent experiments indicate that the degree of isotope fractionation caused by bacterial disproportionation of elemental sulfur may also depend on the availability of reactive metal oxides (Böttcher et al., 2001; Böttcher and Thamdrup, 2001). Though the full biochemical pathway of disproportionation remains unclear, it has been suggested that the first step in disproportionation of thiosulfate involves cleavage into intermediates such as elemental sulfur and sulfite that then further disproportionate into sulfate and sulfide, also producing large fractionations (Cypionka et al., 1998).

As discussed above, isotopic fractionations during sulfate reduction by either pure cultures or natural populations produce large fractionations with an up-

per limit of 49‰. However, in modern marine sediments, larger fractionations of up to 70‰ are observed (Goldhaber and Kaplan, 1974, 1980). In this regard, continued cycles of partial sulfide oxidation followed by disproportionation (the "thiosulfate shunt" of Jørgensen, 1990 and similar reactions) can result in sulfide more depleted in ^{34}S than 49‰, thus explaining the extreme fractionations evident in some modern marine sediments. Similarly large fractionations are also observed in some Neoproterozoic sedimentary rocks (Ross et al., 1995; Logan et al., 1999). Canfield and Teske (1996) argued that these signaled the first significant expression of an oxidative sulfur cycle involving sulfide oxidation and disproportionation of elemental sulfur, indicating an increase in atmospheric oxygen at this time.

5. Preservation of the isotopic record

Dissimilatory sulfate reduction produces large amounts of free H_2S, which mostly reacts with heavy metal ions, especially iron (Berner, 1970, 1984) (Eq. (18)):

$$Fe^{2+} + H_2S \rightarrow FeS + 2H^+ \quad (18)$$

From here, two pathways of pyrite formation have been advocated. First, the metastable iron sulfides may react with intermediate sulfur species such as elemental sulfur or S_x^{2-} to form pyrite (Eq. (19)) (Berner, 1970; Rickard, 1975; Wilkin and Barnes, 1996):

$$FeS + S^0 \rightarrow FeS_2 \quad (19)$$

$$FeS + S_x^{2-} \rightarrow FeS_2 + S_{(x-1)}^{2-} \quad (20)$$

The second pathway involves the oxidation of FeS during which H_2S acts as the oxidizing agent (Eq. (21)) (Drobner et al., 1990; Rickard, 1997; Hurtgen et al., 1999):

$$FeS + H_2S \rightarrow FeS_2 + H_2 \quad (21)$$

The isotopic fractionation associated with pyrite formation from dissolved sulfide at low temperatures is <1‰ (Price and Shieh, 1979; Böttcher et al., 1998). Thus, intact isotopic signatures of biogenic sulfate reduction are preserved in metal sulfides in sediments. As subsequent sulfur remobilization is minimal before lithification and because most geological processes involve little sulfur transport, this primary isotopic signature can be conserved in sedimentary rocks to high metamorphic grades and through vigorous deformation, except in sulfurous magmatic and hydrothermal settings. So, $\delta^{34}S$ patterns of sedimentary pyrites should reflect whether or not biological S-isotopic fractionation occurred in contemporaneous marine environments, allowing the early history of sulfur metabolism to be deciphered.

6. Non-biological sulfur isotopic fractionation

While it is true that microbial sulfate reduction produces large spreads of $\delta^{34}S$ values in sedimentary pyrites, the converse may not always apply because nonbiological sulfate reduction in hydrothermal fluids and other thermal settings can also produce large fractionations between sulfate and sulfide. The consideration of these processes is important because ancient sedimentary rocks billions years old have often experienced thermal alteration. Hence, thermal processes can add non-biologically fractionated sulfides that may mask the primary isotopic signature. So, in order to understand the antiquity of microbial sulfate reduction, we must clarify the genetic history of pyrites in ancient sedimentary rocks. This can only be done by the integration of isotopic data with detailed geochemical and geological observations.

S-isotopic fractionations during sulfate reduction in thermal systems are primarily controlled by the temperature and pH of the fluids (Ohmoto and Lasaga, 1982). Several mechanisms can produce non-biologically fractionated sulfides. Here we briefly discuss sulfate reduction by Fe^{2+}-bearing minerals, hydrolysis of SO_2 and thermochemical sulfate reduction by hydrocarbons.

6.1. Hydrothermal sulfate reduction by Fe^{2+}-bearing minerals

When hydrothermal fluids containing sulfate circulate through surrounding rocks, inorganic reduction of sulfate to sulfide can occur at near-neutral pH and

temperatures >200 °C when Fe^{2+}-bearing minerals are available, according to the following reaction:

$$8Fe^{2+} + 10H^+ + SO_4^{2-} \rightarrow 8Fe^{3+} + H_2S + 4H_2O \quad (22)$$

It has been suggested that the S-isotopic fractionation during sulfate reduction by Fe^{2+}-bearing minerals might be controlled by an equilibrium isotopic effect that is a function of temperature, with fractionation ranging from 20‰ at 300 °C to 30‰ at 200 °C (Ohmoto and Lasaga, 1982; Ohmoto and Goldhaber, 1997). In this case, the isotopic compositions of sulfates in equilibrium with sulfides in the hydrothermal system would become much heavier than that of initial sulfates, potentially allowing easy recognition. More detailed information about non-biological sulfate reduction in hydrothermal systems can be found in Ohmoto and Goldhaber (1997).

6.2. Magmatic hydrolysis of SO_2

Felsic magmas tend to produce somewhat oxygenated aqueous fluids in which oxidized sulfur species may be reduced inorganically. At temperatures >400 °C, the dominant oxidized sulfur species in the fluids would be SO_2 rather than dissolved sulfate (Cameron and Hattori, 1987; Ohmoto and Goldhaber, 1997). During ascent and cooling of such magmatic fluids, H_2S and H_2SO_4 are produced by hydrolysis of SO_2 at temperatures <400 °C, according to Eq. (23):

$$4SO_2 + 4H_2O \rightarrow H_2S + H_2SO_4 \quad (23)$$

This will induce isotopic fractionation (Ohmoto and Goldhaber, 1997). The isotopic difference between H_2S and H_2SO_4 may be controlled initially by the kinetic isotopic effect that is likely to be smaller than the equilibrium fractionation factor. As temperatures drop below 400 °C, H_2S would be depleted in ^{34}S by 15–20‰ relative to sulfate (Ohmoto and Goldhaber, 1997).

6.3. Thermochemical sulfate reduction by hydrocarbons in diagenetic environments

This process involves the non-biological reduction of dissolved sulfate with solid, liquid or gaseous hydrocarbons at elevated temperatures in deeply buried sedimentary rocks (Machel, 2001). The overall reaction, which requires no external catalyst and proceeds slowly over many thousands to millions of years, is:

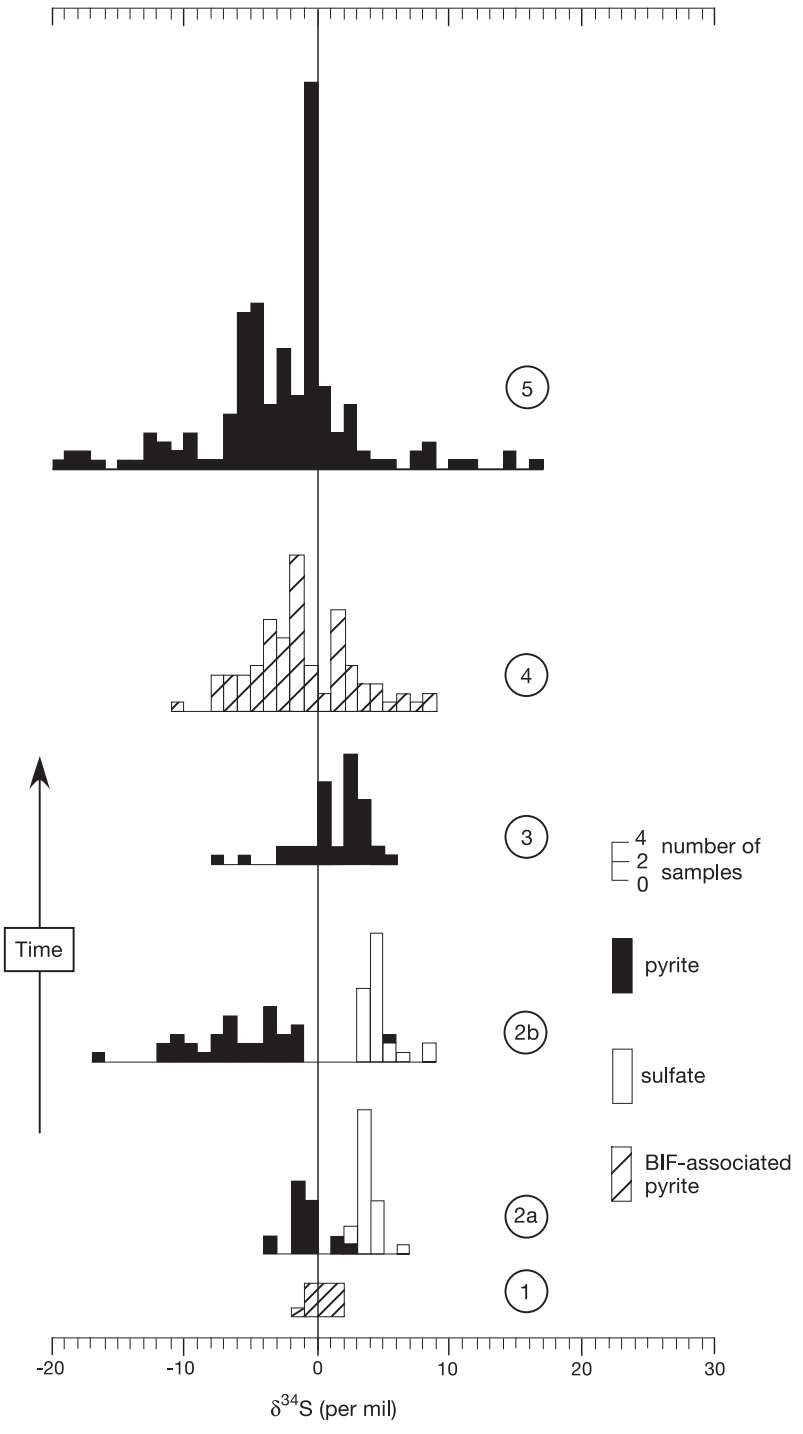
$$4CH_2 + 3SO_4^{2-} + 6H^+ \rightarrow 3H_2S + 4HCO_3^- + 4H^+ \quad (24)$$

The dissolved sulfate is usually derived from soluble evaporitic sulfate minerals such as gypsum or anhydrite, the dissolution of which enriches brines in Ca^{2+} ions. Because of this and the large amounts of bicarbonate also generated, thermochemical sulfate reduction is usually characterized by the deposition of coarsely crystalline secondary carbonate minerals, principally calcite, as replacements of the original sulfate minerals (Machel, 2001). Sulfur isotopic fractionation between parent sulfate and daughter sulfides can be considerable (Machel et al., 1995), due to the large isotope effect at moderate temperatures. However, as dissolved sulfate concentrations control the reaction and as it occurs in deep settings under closed hydrological conditions, the reaction usually proceeds to completion and bulk sulfide $\delta^{34}S$ therefore closely approximates that of the parent sulfate (Machel et al., 1995).

7. Archean S-isotopic record

We first summarize knowledge of the Archean sulfur isotopic record prior to the publication of Shen et al. (2001), and then focus on our recent data from

Fig. 6. The S-isotope record of sulfide and sulfate in Archean sedimentary rocks. Data source: 1: Pyrites in Banded Iron Formation (BIF) from Isua of ~3.8 Ga, West Greenland (Monster et al., 1979); 2a: Sulfides and sulfates from the barite deposits of ~3.47 Ga, North Pole, Australia (Lambert et al., 1978); 2b: Sulfides and sulfates from the barite deposits of ~3.47 Ga, North Pole, Australia (Shen et al., 2001); 3: Pyrites in black shales of ~2.7–2.8 Ga from greenstone belts of the Yilgarn Block, western Australia (Donnelly et al., 1977); 4: Pyrites in Michipicoten and Woman River Iron Formations of ~2.7 Ga, Superior Province, Canada (Goodwin et al., 1976); 5: Pyrites in sedimentary rocks of ~2.7 Ga from the Spring Valley and Jimmy Members of the Manjeri Formation, Belingwe Greenstone Belt, Zimbabwe (Grassineau et al., 2000).


5
4
3
2b
2a
1
Time
4
2
0
number of samples
pyrite
sulfate
BIF-associated pyrite
-20
-10
0
10
20
30
δ34S (per mil)

the North Pole barites. We will use this updated record to discuss the antiquity of sulfate-reducing prokaryotes and the implications for early Archean ecosystems.

The oldest terrestrial S-isotopic records come from highly metamorphosed and deformed ferruginous rocks resembling banded iron-formations from the Isua Supracrustal Belt, Greenland (~ 3.8 Ga), which show a narrow range with a mean value of $+0.5 \pm 0.9$‰ (Monster et al., 1979) (Fig. 6(1)). With few exceptions, sedimentary sulfides between 3.8 and 2.8 Ga are characterized by similarly narrow ranges about magmatic values, with isotopic compositions of -5‰ to $+10$‰ around an inferred $\delta^{34}S$ of coeval seawater sulfate of about $+3-5$‰ (Fig. 6). In contrast, sedimentary sulfides in the ~ 2.7-Ga-old Michipicoten and Woman River Iron Formations of Canada are distinctly shifted to the negative, with $\delta^{34}S$ values as low as -17.5‰ and a large spread (Goodwin et al., 1976) (Fig. 6(4)). The $\delta^{34}S$ distributions of these sedimentary sulfides thus provide strong evidence that microbial sulfate reduction had evolved by 2.7 Ga (Goodwin et al., 1976; Schidlowski et al., 1983). New S-isotope data from 2.7 Ga rocks of the Manjeri Formation in the Belingwe Belt, Zimbabwe are also characterized by a wide range of $\delta^{34}S$ values from -19.9‰ to $+16.7$‰, thus reinforcing the previous conclusion that microbial sulfate reduction had evolved by 2.7 Ga (Grassineau et al., 2000; Nisbet and Sleep, 2001) (Fig. 6(5)). Similarly wide isotopic ranges are observed in sedimentary rocks spanning the Archean–Proterozoic boundary (Hayes et al., 1992; Kakegawa et al., 1999). By ~ 2.3 Ga, large fractionations of up to 40–45‰, typical of microbial sulfate reduction with non-limiting sulfate, are observed in the upper Transvaal Supergroup of South Africa (Cameron, 1982). Such large fractionations of $\delta^{34}S$ for sedimentary sulfides are then general in the Proterozoic isotopic record until approximately 1.0 Ga. The abrupt and persistent increase in S-isotopic fractionation around 2.3 Ga might signal an increase in sulfate concentration in the early Proterozoic oceans (Cameron, 1982). From ~ 1.0 Ga on, the continuous record of very large fractionations (>45‰) indicates that the complete modern sulfur cycle involving reduction, re-oxidation and disproportionation was fully established (Canfield and Teske, 1996).

7.1. Interpretations of the early Archean isotopic record

Though it is generally agreed that microbial sulfate reduction had evolved by 2.7 Ga, the reasons for the small fractionations and narrow distributions of S-isotope values from older rocks remain controversial (e.g., Paytan, 2000). The minimally fractionated early Archean sulfides have been widely attributed to either biological sulfate reduction at low sulfate concentrations (< 1 mM), implying poorly oxygenated oceans, or a non-biological volcanogenic origin, implying sulfate-reducers had not yet evolved (Cameron, 1982; Walker and Brimblecombe, 1985; Habicht and Canfield, 1996; Canfield et al., 2000; Habicht et al., 2002). By contrast, it has been argued that the small fractionations of early Archean sulfides were formed by vigorous biological sulfate reduction in warm, oxygenated and sulfate-rich Archean oceans (Ohmoto and Felder, 1987; Ohmoto et al., 1993). According to this model, high rates of sulfate reduction triggered by high ocean temperatures would have resulted in complete sulfate depletion near the sediment–water interface, inducing an effective closed system in which minimally fractionationed sulfides would have been produced (Ohmoto and Felder, 1987; Ohmoto et al., 1993). However, our current understanding of the factors controlling isotopic fractionation during sulfate reduction does not support for this model, nor does empirical evidence derived from recent laboratory and field studies.

Earlier studies with a single organism over a narrow temperature range suggested that the specific rate of sulfate reduction increases with increasing temperature (Harrison and Thode, 1958; Kemp and Thode, 1968). However, in contrast to those data, a large survey of the relations between sulfate reduction rates and temperature clearly show that specific rates of sulfate reduction for a wide variety of sulfate-reducers are not coupled to temperature (Canfield et al., 2000). Though different sulfate-reducing microorganisms grow over a broad range of environmental temperatures from -2 °C to around 105 °C (Jørgensen et al., 1992; Sagemann et al., 1998), individual species of sulfate-reducers have a much narrower temperature range (e.g., Llobet-Brossa et al., 1998; Ravenschlag et al., 2000). Accordingly, temperature may be important in regulating the species composition of sulfate-reducing communities in different

environments rather than the dominant factor controlling reduction rates.

As discussed above, both pure cultures and natural populations clearly demonstrate that there is little genetic relationship between high rates of sulfate reduction and small fractionations (Fig. 5). Rather, large fractionations of >15 ‰ are often observed in natural population studies of microbial mats that support the highest rates of sulfate reduction (Habicht and Canfield, 1996). Further, at any given sulfate reduction rate, there is a wide range of fractionation values (Chambers and Trudinger, 1979). Thus there is little reason to infer that higher specific rates should result in fractionations near 0 ‰ at higher temperatures.

Moreover, in modern marine environments, most sulfate reduction and pyrite formation occurs near the sediment surface where sulfate reduction rates are highest and active sulfate-reducers are most abundant (Jørgensen, 1982; Skyring, 1987; Llobet-Brossa et al., 1998; Böttcher et al., 1999). However, only small sulfate depletions are evident in near-surface pore water (e.g., Jørgensen, 1979; Böttcher et al., 1999; Brüchert et al., 2001). A diffusion-reaction diagenetic model applied to a sulfate-rich ocean shows that high sulfate reduction rates do not result in closed-system behavior; rather, high sulfate reduction rates are only associated with rapid organic matter consumption (Canfield et al., 2000). Therefore, with modern levels of seawater sulfate, as assumed in the sulfate-rich Archean ocean model (Ohmoto and Felder, 1987; Ohmoto et al., 1993), high rates of sulfate reduction should not induce closed system behavior, and thus should not mask the normal broad isotope fractionation imposed by sulfate-reducers in sediments. So, all of our current knowledge is markedly inconsistent with the model that minimally fractionated early Archean sulfides were generated by vigorous microbial sulfate reduction in warm oxygenated oceans.

Recent studies indicate that there is no relationship between phylogenetic position, as measured on the 16S rRNA Tree of Life, and isotopic fractionation (Detmers et al., 2001). Even the deepest-branching and thus earliest-arising lineages show fractionations like those of more recently evolved groups. Hence, it is likely that ancient sulfate-reducing organisms fractionated isotopes in a similar manner to their modern counterparts. If so, low fractionations in Archean sulfides would be most consistent with very low concentrations of seawater sulfate (Cameron, 1982; Hayes et al., 1992; Knoll and Canfield, 1998; Canfield and Raiswell, 1999), as that may be the sole circumstance under which their modern relatives impart such a negligible overall fractionation. However, there are relics of some isolated sulfate-rich basins in the early Archean geological record (e.g., Lambert et al., 1978; Buick and Dunlop, 1990). In these, the barrier of low sulfate concentration would have been removed, potentially allowing microbial sulfate reduction to have proceeded in recognizable fashion, provided of course that organisms with such metabolism had evolved. So, these isolated sulfate-rich sedimentary environments are promising targets for detecting the metabolism of microbial sulfate reduction early in Earth history.

8. North Pole

The best-known site of sulfate-rich sedimentation in the early Archean is at North Pole in northwestern Australia (Fig. 7). The rocks there belong to the >3.46-Ga (Thorpe et al., 1992a,b; McNaughton et al., 1993; Buick et al., 1995) Warrawoona Group, a predominantly basaltic unit about 10 km thick that is remarkably well-preserved considering its great age. Undeformed pillows, amygdales and varioles are common in the basalts, indicating that the rocks have generally undergone only low-strain brittle deformation (Dunlop and Buick, 1981). Pillow margins from drill-core contain prehnite and pumpellyite or epidote and actinolite as prograde mineral assemblages, indicating peak metamorphic grades of prehnite-pumpellyite to lowermost greenschist facies (Dunlop and Buick, 1981). Dips are generally low, as the rocks have been arched into a broad (~ 10 km wide) structural dome centered upon a slightly younger (~ 3.46 Ga) monzogranite intrusion. Since the early Archean, they have undergone only shallow burial by late Archean flood basalts of the Fortescue Group and some further mild deformation associated with the waning stages of Fortescue tectonism. The exposure is good, because successive Neogene uplifts resulting from impingement between the Australian and Asian plates have neutralized the effects of continent-wide deep weathering and peneplanation.

Sediments occur as thin (5–50 m) interbeds within the dominantly basaltic succession of the Warrawoona Group. Though there are nine significant sedimentary

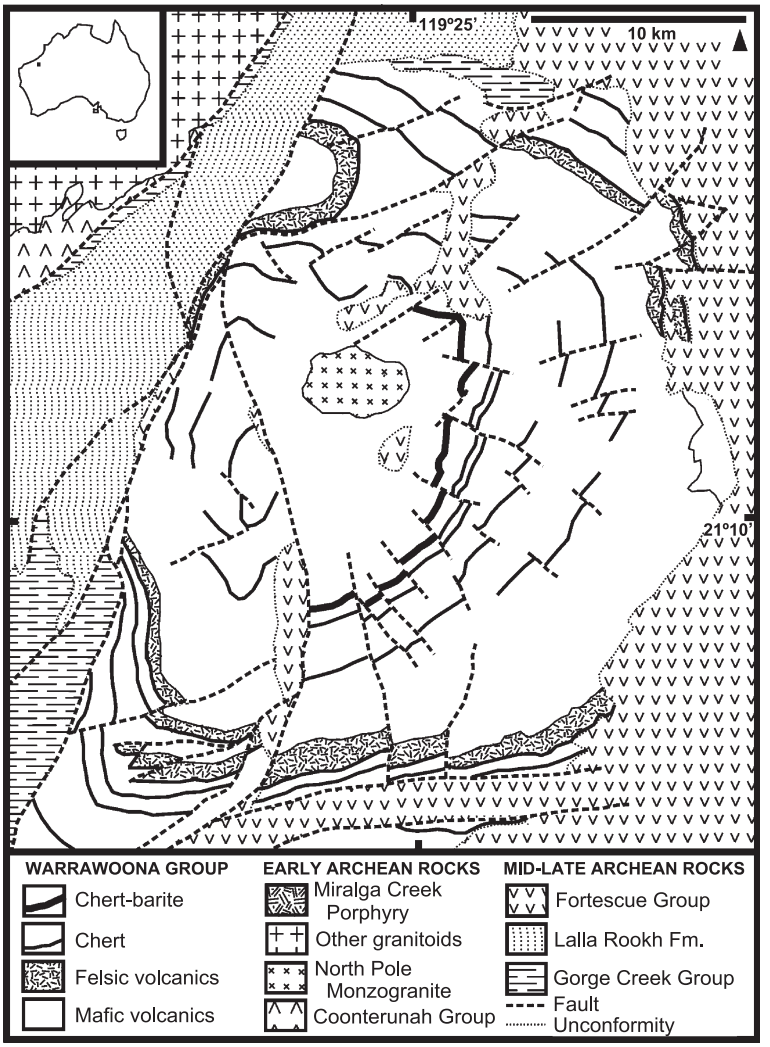


Fig. 7. Geological map of the North Pole Dome showing the location of the chert-barite unit, the lowermost sedimentary interval in the ~ 3.47 Ga Warrawoona Group.

interludes, only six contain evidence of sulfate sedimentation and just two preserve original sulfate (Buick and Barnes, 1984). The best developed is the lowermost, the basal unit of the Dresser Formation, which is ~ 40 m thick (Fig. 8). In this, and in all other sedimentary horizons within the Warrawoona Group at North Pole, original sedimentary minerals have been largely replaced by hydrothermal silica, now chert. However, primary textures and structures are in places perfectly preserved, defined by inclusions of original iron–titanium oxides, by metamorphic sericite and chlorite which has replaced original volcanic glass, or by microinclusions of dolomite after original carbonates (Buick and Barnes, 1984). Large sulfate crystals are now composed of barite ($BaSO_4$). Small sulfate crystals have been totally replaced by microcrystalline silica, now microquartz (Fig. 9G). Some of those intermediate in size have a barite core and microquartz rind, and all of the large barite crystals have been silicified marginally to a depth of 1–2 mm (Fig. 9E).

From textural and structural evidence in the chert and the surrounding basalt, the depositional environment was shallow subaqueous, indicated by the presence of wave ripples, well-sorted cross-beds and

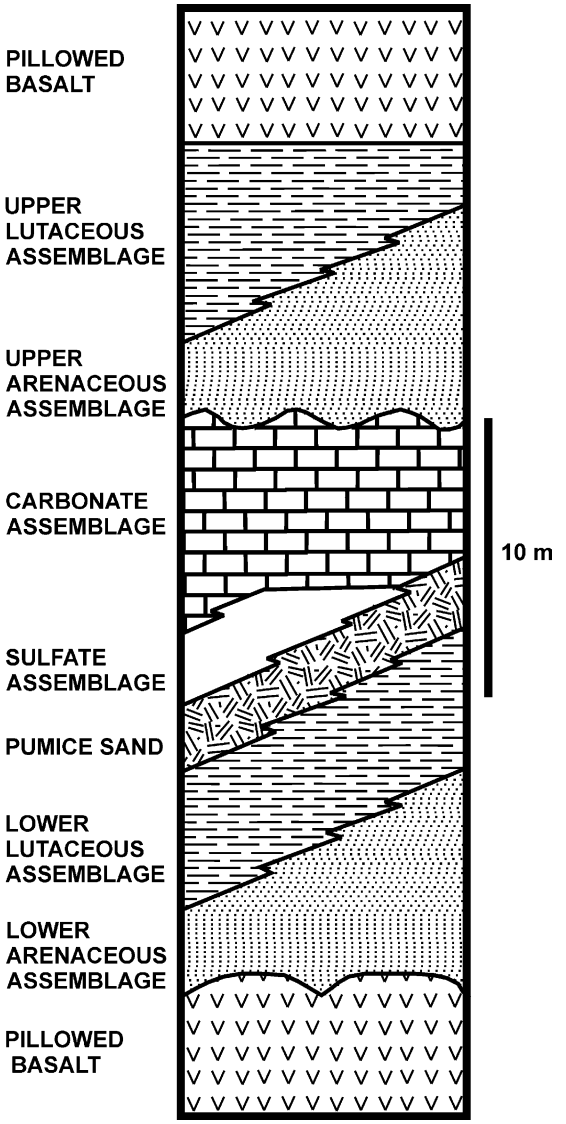


Fig. 8. Schematic stratigraphic section of the North Pole chert-barit unit, showing the relationship between the former gypsum evaporites (sulfate assemblage), other evaporative facies (carbonate assemblage) and a semi-permeable beach barrier of pumice sand that separated the sea from the evaporite mud-flats and ponds.

scoriaceous tops to pillowed basalt flows (Groves et al., 1981). Intermittent exposure was marked by formation of desiccation breccias. Given the widespread outcrop of some of the sedimentary horizons in the succession (over 150 km before unfolding), the depository was evidently marginal marine (Fig. 10). The sulfate deposits apparently formed in back-barrier lagoons separated from the ocean by beaches and bars of rafted pumice (Groves et al., 1981). Surrounding the sulfate ponds were tidal flats composed of either carbonate sand or volcanogenic mud, in both of which diagenetic sulfate crystals and nodules precipitated. Interfacial angle measurements on the diagenetic crystals show that they were initially composed of gypsum ($CaSO_4 \cdot 2H_2O$) which grew before lithification, indicated by their cores of incorporated sediment (Lambert et al., 1978; Buick and Dunlop, 1990). The elongate prismatic form of the crystals, most of which are isolated individuals but arranged into rosettes in coarser sediment (Fig. 9G), and their pristine angular terminations without any signs of resorption indicate that the gypsum was not metastable and was in thermodynamic equilibrium with surrounding pore-waters (Buick and Dunlop, 1990).

Primary sulfate crystals precipitated in the back-barrier brine ponds, forming lenticular beds up to 0.5 km in lateral extent and up to 10 m thick (Fig. 9A). Individual layers of sulfate crystals (now dark grey barite) are up to 15 cm thick (Fig. 9B), composed of bottom-nucleated subradiating fans of bladed hemipyramids (Buick and Dunlop, 1990). Where beds were draped by detrital sediment, the contrasting contact preserves the interfacial angles of the original sulfate crystals (Fig. 9C). When measured with a universal stage, their angles indicate that these particular draped crystals precipitated as gypsum (Lambert et al., 1978; Buick and Dunlop, 1990). Though this does not preclude other primary sulfate crystals from forming as barite, the mere presence of *any* primary gypsum is highly significant. That the gypsum was indeed primary is confirmed by the presence on the draped interfaces of broken, eroded and rounded crystals filling interstices between protruding crystal fans.

Barite replacement of gypsum evidently occurred soon after diagenesis began and shortly after burial by the overlying basalts, shown by the perfect preservation of crystal morphologies of the highly soluble phase gypsum. Substitution of Ba^{2+} ions for Ca^{2+} will occur spontaneously when gypsum is bathed by fluids enriched in dissolved barium (J.S.R. Dunlop, unpublished data), because the ions are similar in size and charge but barite is much less soluble than gypsum. The most likely barium source was hydrothermal brines that percolated into the porous sediments from the surrounding hot basalt pile. Because of the very

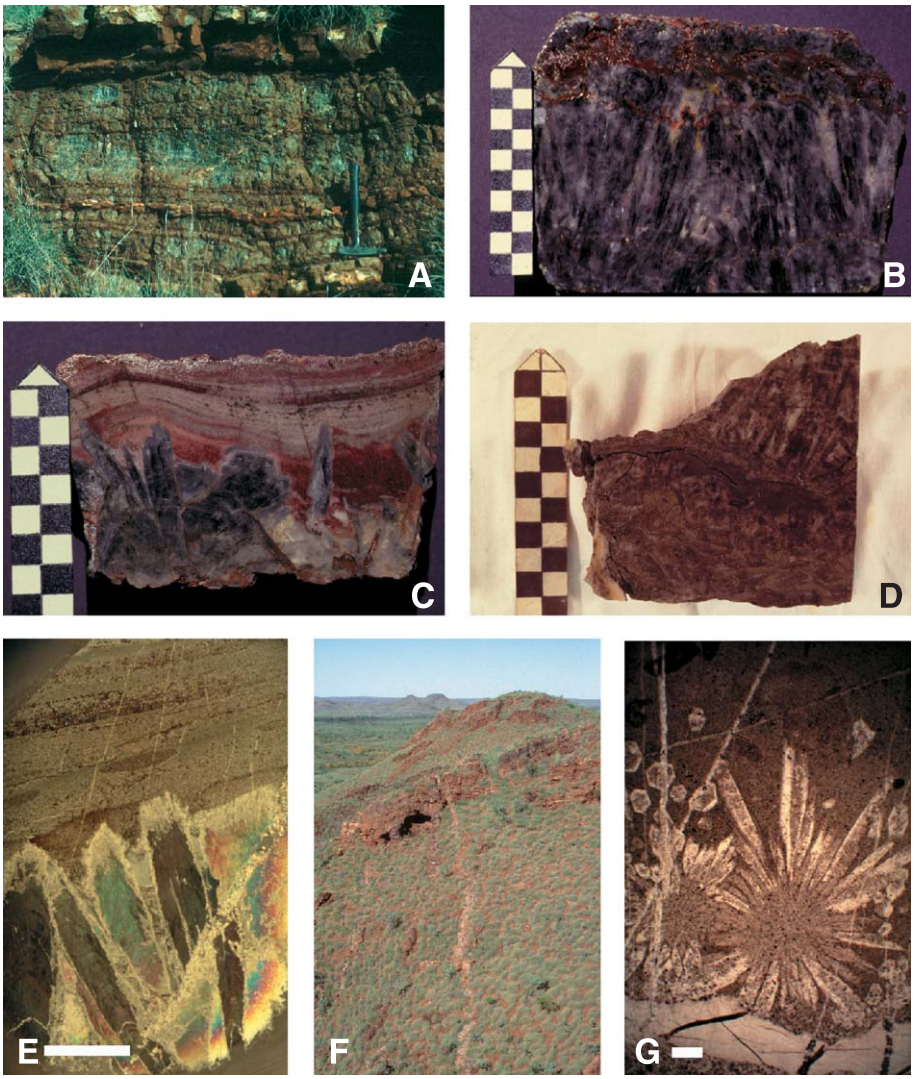


Fig. 9. Sulfate deposits at North Pole: (A) beds of barite (hammer at right for scale); (B) sawn slab of bedded barite showing bottom-nucleated, radiating fans of bladed crystals (centimetre scale); (C) sediment-draped barite crystals showing interfacial angles typical of gypsum (centimetre scale); (D) colloform laminae of pyrite (bronze) separating beds of barite (dark grey) from a fresh mine sample (centimetre scale); (E) thin section of sediment-draped barite show in marginal silicification of barite crystals and incongruence between original crystal faces and current barite crystals (scale bar = 10 mm); (F) vein of pale grey barite transecting dark grey bedded barite; (G) rosette of acicular crystals of diagenetic gysum in sandy interbed within bedded barite (scale bar = 1 mm).

low solubility of barium ions in the presence of sulfate, the two species cannot have been transported in the same fluid, indicating that the sulfate was primary, evaporitic and derived directly from contemporary seawater. As baritization of gypsum requires excess sulfate, there being about 30% difference in unit cell volume between the two phases, this may have been sourced from the diagenetic gypsum crystals that are now replaced by quartz, from the margins of the primary gypsum crystals that are now replaced by quartz, or from residual evaporitic pore fluids.

Beneath and truncating the bedded barite lenses are barite veins up to 5 meters across and 200 meters deep (Fig. 9F). The barite in these is pale grey to white, forming undulose layers parallel to the walls of the veins that are interspersed with layers of amorphous

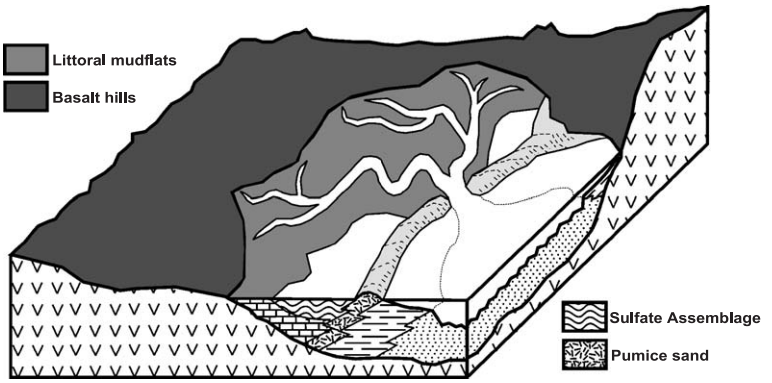


Fig. 10. Schematic reconstruction of North Pole evaporative sulfate depositional environment.

white or grey chert. Vein barite has a different crystal morphology to the bedded barite, consisting of platey pinacoids with well-developed planar prismatic terminations. The veins may represent the hydrothermal conduits where warm, barium-rich fluids mixed with surficial sulfate-rich brines. Some sulfate remobilized from sedimentary gypsum could have also contributed to the growth of barite in the veins.

Usually, bedded barite layers are separated by thin (~ 1 cm) wavey partings of iron oxides which, where freshly exposed, are composed of undulose or crescentic pyrite laminae 1–2 mm thick (Figs. 9D and 11C). As well, individual barite crystals contain sulfidic microinclusions, mostly pyrite but some arsenopyrite and sphalerite, aligned along growth faces of the original gypsum crystals (Fig. 11A,B). This mineralogical relationship between microscopic pyrites and the original gypsum crystals clearly indicates the syngenesis of sulfate and sulfides, and confirms that most sulfate is residual from the precursor gypsum. The microscopic pyrites are ~ 50 μm in size, comprise less than 1% of the rock and are closely associated with kerogen inclusions (Buick and Dunlop, 1990). Also within the barite crystals are H_2S-containing fluid inclusions ~ 10 μm in size that apparently formed during crystal growth (Rankin and Shepherd, 1978). Barite in transecting veins contains fine, disseminated pyrites, but lacks pyrite laminae, sulfurous fluid inclusions and kerogen inclusions.

We examined the sulfur isotope systematics of all accessible phases, i.e., the bedded barite, the vein barite, the pyrite laminae and the pyritic microinclusions. Previous studies (Lambert et al., 1978) had analyzed the first three of these, along with some late galena mineralization, but did not investigate the microscopic pyrites. Before performing S-isotope analysis, we first examined the mineralogy under a polarizing microscope and choose those bedded barite samples in which gypsum crystal morphologies and pyrite laminae and micro-inclusions were best preserved. Indeed, we deliberately sampled specimens from which interfacial angles had been measured, to ensure that the sulfate was derived from gypsum.

8.1. S-isotopic results and discussion

Our $\delta^{34}S$ data for sulfates are consistent with previous analyses by Lambert et al. (1978), mostly falling between +3.2 ‰ and +5 ‰ with an average of +4.3 ‰ (Fig. 6(2b)). Previous data from the macroscopic pyrite laminae revealed an average $\delta^{34}S$ value of −0.9 ‰ (Lambert et al., 1978) (Fig. 6(2a)). Our isotopic data are similar though slightly more ^{34}S-depleted, between −1.0 ‰ and −3.5 ‰ with an average of −2.4 ‰ (Fig. 6(2b)). While these pyrites could represent unfractionated volcanogenic sulfur, they are consistently more ^{34}S-depleted than is typical of younger volcanogenic sulfide deposits (Ohmoto and Goldhaber, 1997). However, they are not sufficiently ^{34}S-depleted to be definitely biological, so their origin is still equivocal (Buick and Dunlop, 1990).

By contrast, previously neglected microscopic sulfides in the bedded barites are highly ^{34}S-depleted (Fig. 6(2b)), with fractionations relative to coexisting sulfate ranging from 21.1 ‰ to 7.4 ‰, with a mean of 11.6 ‰. As discussed above, non-biological processes

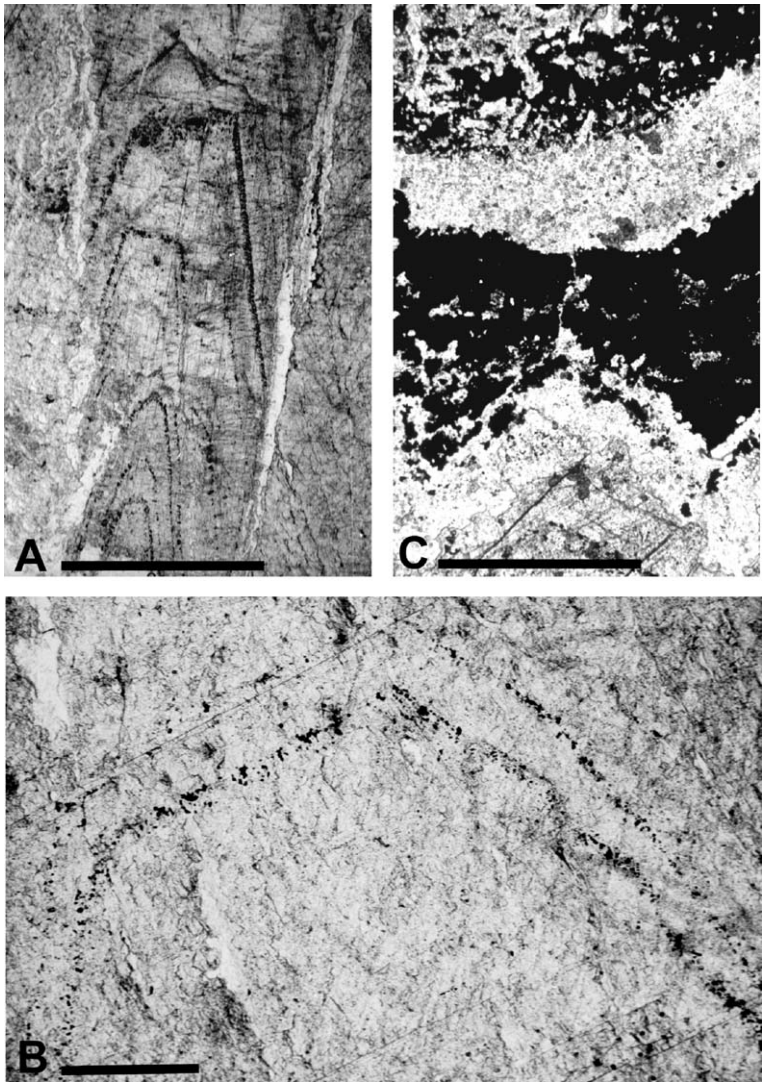


Fig. 11. The relationships between sulfur species in the North Pole barite deposit: (A and B) microcrystalline pyrite included within barite crystals but aligned along growth faces of the original sulfate crystals (scale bar = 1 mm); (C) opaque pyrite lamination between barite beds and draping over individual barite crystals (scale bar = 1 mm).

can, in principle, produce isotopic fractionations of this magnitude. For example, hydrolysis of SO_2 to sulfate and sulfide in relatively oxidizing magmatic fluids will cause isotopic fractionation (Ohmoto and Goldhaber, 1997). However, the rocks surrounding the North Pole deposits are basaltic (Buick and Dunlop, 1990) and so would have generated reduced rather than oxidized magmatic fluids. Also, there is no geological evidence of magmatic contributions to barite deposition, because no vent orifices or sinter terraces have been found within the bedded barite units, nor have any cross-cutting zones of particularly advanced alteration been identified in directly underlying rocks (Buick and Dunlop, 1990). Furthermore, ^{34}S-depleted sulfides precipitated with sulfates from oxidized Archean magmatic fluids are usually associated with pervasive hematite alteration (Cameron and Hattori, 1987), absent from the North Pole deposits.

Hence, the hydrolysis of SO_2 from a felsic magmatic source is an unlikely explanation for our results.

It is also conceivable that a recirculating hydrothermal system with sulfate entrained from the evaporating brine ponds could have produced ^{34}S-depleted sulfides during partial sulfate reduction in hot underlying basalts. Inorganic reduction of sulfate to sulfide by ferrous minerals can occur at near-neutral pH and temperatures >200 °C (Eq. (21)). A spectrum of isotope values could, in principle, result from variable sulfate depletion or fluctuating temperatures during hydrothermal sulfate reduction. However, the maximum hydrothermal temperature allowed by the mineralogical assemblages at North Pole is ~ 300 °C, which would produce from sulfate at +4‰ sulfides at least as light as −16‰. By contrast, most of our sulfide data fall between −5‰ and −10‰, outside the range expected for hydrothermal reduction of early Archean marine sulfate. Importantly, had sulfate come from hydrothermal fluids, the $\delta^{34}S$ values of some sulfate crystals should be much heavier than +4‰. The low solubility of barite requires that Ba^{2+} would have scavenged sulfate very efficiently from the hydrothermal fluids. Accordingly, the resulting barite should record highly variable and much heavier $\delta^{34}S$ values than the initial sulfate. However, the nearly uniform $\delta^{34}S$ values for the North Pole barites argue against such a scenario.

Because traces of hydrocarbons have been found in the North Pole deposit (Buick et al., 1998), thermochemical sulfate reduction must also be considered as a possible source of the isotopically fractionated microcrystalline pyrites. However, the moderate spread and large degree of isotopic fractionation is atypical of sulfides of thermochemical origin, as is the absence of coarse secondary calcite crystals. Moreover, no residues of mobile hydrocarbons such as pyrobitumen or petroleum-bearing fluid inclusions have been found in or near the host sulfate crystals (Dutkiewicz and Ridley, 2003), but thermochemical sulfate reduction requires an intimate association between such reactants. Lastly, the position of the pyrite microcrystals within sulfate crystals, rather than on their exterior, is antithetic to the expected thermochemical relationship. So, a thermochemical isotopic fractionation during diagenesis or metamorphism seems rather unlikely.

Regardless of the S-isotopic data themselves, petrographic relationships provide strong evidence for the genesis of the microscopic pyrites. The original sulfate mineral was gypsum, not anhydrite or barite, the two typical magmatic and hydrothermal sulfates. Gypsum is only stable below ~ 60 °C (Hardie, 1967), so the alignment of the microscopic sulfides along the crystal faces of the original gypsum (Fig. 11) demonstrates that these sulfides were formed at relatively low temperatures along with their host mineral. These microscopic sulfides were, therefore, formed before baritization, the earliest known hydrothermal event at North Pole (Buick and Dunlop, 1990). We thus find no compelling evidence for a magmatic, hydrothermal or thermochemical origin for the microscopic sulfides in the bedded barites at North Pole.

The microscopic sulfides in bedded barites at North Pole are intimately associated with organic carbon in the form of kerogen (Buick and Dunlop, 1990), the principal electron donor for biological sulfate reduction. As is typical for microbial processes of sulfate reduction, the $\delta^{34}S_{sulfide}$ values, from −1.3‰ to −16.8‰, show a general negative trend relative to the parent sulfate and a wide spread about the mean. In addition, isotopic fractionations are within the range observed for modern sulfate-reducing microbes metabolizing with >1 mM sulfate (Harrison and Thode, 1958), especially for organisms growing under optimal conditions where fractionation values of 10–26‰ are typical (Kaplan and Rittenberg, 1964; Chambers and Trudinger, 1979; Habicht and Canfield, 1996). These features, and the lack of evidence for non-biological sources of highly fractionated sulfide, demonstrate that microbial sulfate reduction had evolved by ~ 3.47 Ga.

Sulfides from the vein barites have a similar spread in isotopic fractionations to the bedded barites, ranging from 16.1‰ to 3.4‰. While the veins may have fed hydrothermal fluids to the sedimentary barite beds (Nijman et al., 1999), the vein sulfides show no evidence for a magmatic, hydrothermal or metamorphic origin. More likely, these sulfides were biogenic, although it is unclear whether they were formed in situ or remobilized from surface environments. However, the absence of sulfurous fluid inclusions in the vein barite suggests the latter.

9. Implications for Archean ecosystems

9.1. Early biological evolution

Microbial metabolic pathways control many of the Earth's biogeochemical cycles, but tracing their evolution has proved difficult. Using isotopic data (Hayes, 1994), geochemical inferences (Buick, 1992) and biomarkers (Summons et al., 1999; Brocks et al., 1999), metabolisms such as oxygenic photosynthesis and methylotrophy can now be traced back to the late Archean. This constrains the timing of some key branching events on the small-subunit ribosomal RNA (SSU rRNA) or whole-genome molecular phylogenies that show the fundamental pattern of evolutionary relationships between all living organisms (Knoll, 1999; Banfield and Marshall, 2000). In order to monitor the progress of primordial evolution, it is particularly important to establish the antiquity of all major lineages and thus to constrain the time available for the radiation of life. For example, C_{27}–C_{29} steranes derived from sterols synthesized by eukaryotes (Brocks et al., 1999), and a strong depletion of ^{13}C, a biogeochemical signature of methanogenic Archaea, in 2.7-Ga-old kerogens (Hayes, 1994) independently place a minimum age on two of the three fundamental biological domains (Fig. 12). Also, the identification of abundant $\geqslant C_{31}$ 2α-methylhopanes derived from 2α-Me-bacteriohopanepolyols, membrane lipids synthesized in large quantities only by cyanobacteria, in ~ 2.7 Ga kerogenous shales of the Jeerinah and Marra Mamba Formations (Brocks et al., 1999) constrains the minimum age of this lineage in the Bacterial domain (Fig. 12).

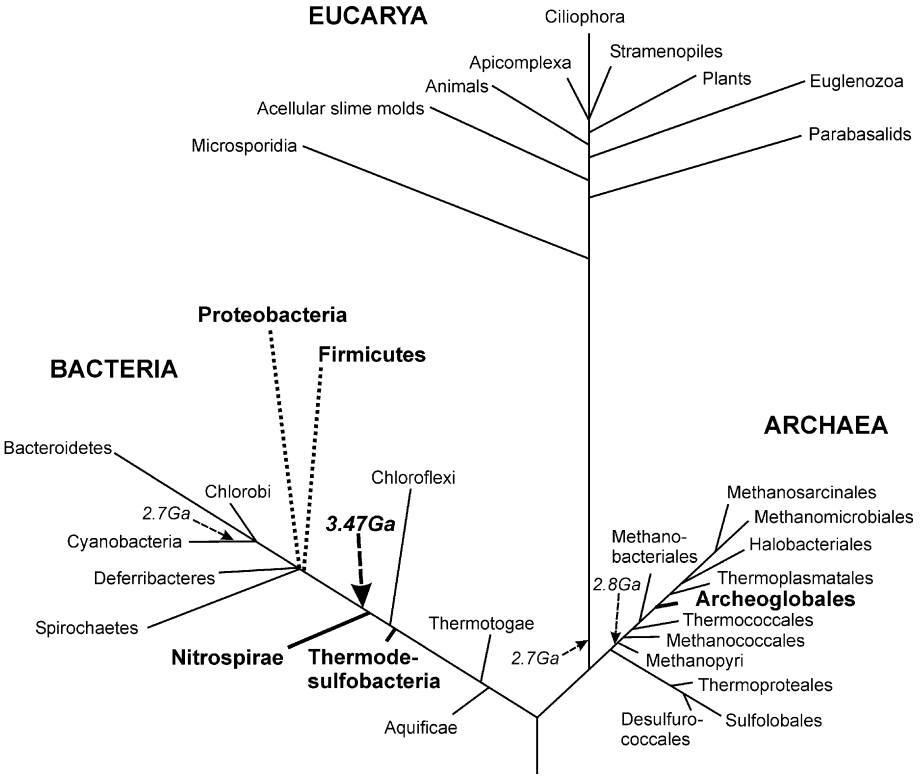


Fig. 12. Short subunit ribosomal RNA phylogenetic tree ("Tree of Life") derived from the Ribosomal Database Project II of Michigan State University full prokaryotic tree determined by Maximum Likelihood methods, 1999; with microbial phyla and orders as in *Bergey's Manual of Systematic Bacteriology*, 2nd edition, Boone, D.R. and Castenholtz, R.W. (Eds.), 2001; showing ancient dates derived from biogeochemical evidence from Shen et al. (2001) with additional data from Knoll (1999). Dotted branches are lineages with mesophilic sulfate reducers; solid thick branches are lineages with thermophilic and hyperthermophilic sulfate reducers only. Note the less conservative placement of the 3.47 Ga date for dissimilatory sulfate reduction as compared to Shen et al. (2001), based on new data for lateral genetic transfer of *dsr* between lineages.

The phylogenetic positions of sulfate-reducing organisms, as revealed from comparisons of SSU rRNA, are widespread through the Archaeal and Bacterial domains (Devereux and Stahl, 1993; Stetter, 1996) (Fig. 12). Among the Archaea, the only known sulfate-reducers are hyperthermophiles with optimal growth temperatures above 80 °C and these are restricted to the single genus *Archaeoglobus* (Stackebrandt et al., 1995; Stetter, 1996). Within the Bacteria, the most deeply branching sulfate-reducers belong to the genus *Thermodesulfobacterium* (Stackebrandt et al., 1995; Stetter, 1996; Pace, 1997), which are also hyperthermophiles with an optimal growth temperature around 80 °C. Thus far, sulfate-reducers metabolizing at temperatures below 70 °C are known only from the δ-subdivision of the Proteobacteria (purple bacteria), the Firmicutes and the phylum Nitrospirae (Fig. 12).

Our S-isotopic data from the North Pole barites reveal that microbial sulfate reduction had evolved by 3.47 Ga. The temperature at which microbes reduced sulfate can be constrained from the characteristics of the bedded barite. Because the original sulfate mineral was gypsum, not its high temperature equivalent anhydrite, the evaporating brine must have been cooler than ~60 °C (Hardie, 1967). Moreover, because the presence of chloride ions lowers the gypsum-anhydrite transition temperature substantially (Holland, 1984), the brine was probably much cooler than this maximum permissible temperature. Seawater, from which the brine was evidently derived, was already saline by the early Archean because halite pseudomorphs are preserved in contemporaneous marine sediments (Boulter and Glover, 1986; Westall et al., 2001). Thus the sulfate-reducing organisms at North Pole must have been mesophiles or, at most, moderate thermophiles. Given our current knowledge of the phylogenetic distribution of thermal adaptations among sulfate-reducers, our findings from the North Pole barites indicate a minimum age of 3.47 Ga for a position immediately above the branching point of the hyperthermophilic *Thermodesulfobacterium* lineage in the Bacterial domain (Shen et al., 2001). This placement is necessarily tentative, as deeper-branching mesophilic sulfate-reducers may be discovered, but even so it represents the oldest evolutionary event thus far dated on the Tree of Life.

The placement is also conservative, because it is at the lowest possible point on the phylogenetic tree consistent with mesophilic sulfate reduction. However, this metabolism may well have arisen elsewhere on the phylogenetic tree requiring a recalibration of evolutionary history. Indeed, recent genetic data (Klein et al., 2001) indicate that the gene for dissimilatory sulfite reductase (*dsr*), the essential enzyme catalyzing the key energy-conserving step in the microbial sulfate reduction metabolic pathway (Fig. 2), has undergone significant lateral transfer between lineages. In particular, the *dsr* gene of the Archaeal hyperthermophile *Archaeoglobus* appears to have been derived from a Bacterial donor, as does that for the similarly hyperthermophilic Bacteria *Thermodesulfobacterium*. As the *dsr* phylogenetic tree is evidently rooted in the thermophilic (optimum growth at 65 °C) *Thermodesulfovibrio* lineage, it would appear that the origin of sulfate reduction thus resides within the medially branching Nitrospirae. If so, then the dated node provided by the North Pole data can be moved up the Tree of Life to a position between the divergence points of the Nitrospirae and the mesophilic sulfate-reducers (δ-Proteobacteria, Firmicutes). This would imply that a good deal of microbial evolution occurred during the first billion years of Earth history prior to the advent of mesophilic sulfate reduction.

9.2. The Early Archean atmosphere and oceans

Several geological indicators including the sedimentary abundance and distribution of the redox-sensitive elements Fe and U, the absence of red beds, and the prevalence of Fe-depleted paleosols suggest that the Archean atmosphere contained little oxygen (Holland, 1984). The recent findings of abundant detrital pyrite and siderite in Archean fluvial siliciclastic sediments (ca. 3250–2750 Ma) from the Pilbara Craton in Australia provide even clearer evidence that the Archean atmosphere was much less oxygenated than at present (Rasmussen and Buick, 1999). But in the past few years, it has also been argued with vigor and ingenuity that the Archean atmosphere was in fact oxic and not much different from today's (Ohmoto, 1997). Clearly, the oxidation state of the early Archean atmosphere remains controversial.

As the sulfur cycle is involved in atmospheric oxygen regulation, Precambrian sulfur isotopic records over geological time have been used to help understand the evolution of atmospheric oxygenation (Hayes et al., 1992; Canfield and Teske, 1996; Knoll and Canfield, 1998; Canfield et al., 2000; Habicht et al., 2002). The link between atmospheric oxygen and seawater sulfate is through the oxidative weathering of metal sulfide minerals on the continents and the subsequent delivery of dissolved sulfate to the oceans. Accordingly, less oxidative weathering of sulfides and thus low concentrations of seawater sulfate would be consistent with lower levels of atmospheric oxygen (Cameron, 1982; Hayes et al., 1992; Knoll and Canfield, 1998). By contrast, a sulfate-rich Archean ocean would provide supportive evidence for high Archean atmospheric oxygen concentrations (Ohmoto and Felder, 1987; Ohmoto et al., 1993).

As discussed above, most Archean sedimentary sulfides older than ~2.7 Ga display low fractionation values, generally less than 10‰. By contrast, our data from the North Pole barite deposit show a large spread of $\delta^{34}S$ values and large S-isotopic fractionations (Fig. 6(2b)), indicating that the metabolic process of microbial sulfate reduction had evolved by 3.47 Ga. The differences between the North Pole deposit and other Archean sedimentary sulfides probably reflect environmental variations. The North Pole evaporite ponds were localized oases of high sulfate concentrations maintained by evaporative concentration and hydrologic semi-isolation, containing organic electron donors derived from an indigenous stromatolitic microbiota (Buick et al., 1981; Buick and Dunlop, 1990). Thus, microbial sulfate reduction could operate under favorable conditions and consequently induce large isotopic fractionations. Accordingly, other Archean sedimentary sulfides characterized by small fractionations may have resulted from microbial sulfate reduction under low ocean sulfate concentrations. Our results thus support models of low atmospheric oxygen during the early Archean.

While converging lines of evidence indicate low sulfate concentrations in early Archean oceans, identifying the sulfate source remains a challenging issue. One possibility is that it was microbial, produced by the green and purple photoautotrophic sulfur-oxidizing bacteria of the Chlorobiaceae and Chromatiaceae. In the presence of light, these anoxygenic photosynthesizers can oxidize H_2S to SO_4^{2-} (Eq. (25)):

$$2HCO_3^- + H_2S \rightarrow 2CH_2O + SO_4^{2-} \quad (25)$$

Such organisms could well have been responsible for accreting the stromatolites interbedded with the North Pole barite. Alternatively, anaerobic chemoautotrophic sulfur-oxidizing microbes such as the proteobacterium *Thioploca* may have transformed sulfide to sulfate using nitrate (e.g., Otte et al., 1999). As sulfate reduction was also anaerobic, using either organic carbon or molecular hydrogen as an electron donor, a complete sulfur cycle could have operated on the early Earth in the total absence of free oxygen. If, however, cyanobacteria were the stromatolite-building organisms, then the sulfur cycle could have more closely resembled its modern form, albeit with aerobic sulfur-oxidizers restricted to mildly oxygenated settings in the photic zone proximal to sources of photosynthetic O_2.

Recent isotopic measurements of the stable isotopes $\delta^{33}S$ and $\delta^{36}S$ from Precambrian sulfides and sulfates show mass-independent fractionation trends, indicating that gas-phase atmospheric reactions also played an important role in the Archean sulfur cycle (Farquhar et al., 2000, 2001). Volcanic emission on the early Earth should have been significantly more vigorous than today, injecting large amounts of SO_2, among other gases, into the atmosphere (Kasting et al., 1989; Kasting, 2001). Photochemical reactions of SO_2, including photolytic oxidation to SO_3 and ultimately to H_2SO_4, generated the mass independent isotopic signatures and would have contributed sulfate to early Precambrian oceans (Farquhar et al., 2000, 2001). However, the quantitative contribution of sulfate from the Archean atmosphere requires further investigation.

It has been argued from North Pole $\delta^{33}S$ and $\delta^{36}S$ data (Runnegar et al., 2002) that the large $\delta^{34}S$ fractionations observed there are not biological, as concluded by Shen et al. (2001). Runnegar et al. (2002) showed that $\delta^{33}S$ and $\delta^{34}S$ of the North Pole barites and associated pyrites do not follow the mass-dependent array (i.e., $\Delta^{33}S = \delta^{33}S - 0.515 \times \delta^{34}S = 0$) defined by Farquhar et al. (2000), but instead plot on a parallel trend with constant $\Delta^{33}S$ values. From this, they concluded that the mass-independent signature (i.e., $\delta^{33}S \neq 0.515 \times \delta^{34}S$) from the North Pole barites was hydrothermally induced at temperatures of about

150 °C. However, though mass-independent fractionation is clearly an important component of the isotopic evolution of the various sulfur species at North Pole, in common with other Archean sulfurous deposits, there are several reasons for doubting that the $\delta^{34}S$ signature of North Pole barites is hydrothermal. First, as discussed above, the rocks surrounding North Pole barites are basaltic which would have generated reduced magmatic fluids, providing conditions inimical to the production of and subsequent reduction of oxidized sulfur species inorganically. Secondly, there is no known hydrothermal mechanism for inducing mass-independent fractionation. Thirdly, other Archean deposits of demonstrably non-hydrothermal origin show similar degrees of mass-independent fractionation to the North Pole deposit. In fact, rather than a hydrothermal origin, the new data presented by Runnegar et al. (2002) are better interpreted as an initial photochemical mass-independent fractionation followed by a large biological mass-dependent fractionation. $\delta^{33}S$ vs. $\delta^{34}S$ values for North Pole barite and associated pyrite plot on a single trend parallel to but displaced below the mass-dependent fractionation line (Farquhar et al., 2000), typical of secondary biological reprocessing of mass-independently fractionated sulfate (Farquhar et al., 2001). Thus, biological mass-dependent sulfate reduction remains the only known process capable of explaining the size and variety of $\delta^{34}S$ fractionations present in the North Pole barites and microcrystalline pyrites.

9.3. Microbial metabolism in the early Archean

Sulfate reduction is a complex metabolic process requiring advanced membrane-bound transport enzymes, proton motive force generation through the activities of ATPase and other proteins involved in charge separation, and the genetic regulation of protein synthesis through DNA and RNA (Widdel, 1988). As such, the oldest evidence of microbial sulfate reduction revealed in the North Pole barite deposit indicates that by 3.47 Ga ago and probably earlier, microbes had already developed many of the critical cellular systems shared by their modern descendants. Thus, early organisms were not just simplified and rudimentary versions of their modern counterparts, but displayed comparable biochemical and cellular sophistication to that of extant prokaryotes.

Furthermore, the demonstration of microbial sulfate reduction in the North Pole barites provides the earliest indication of a specific metabolic pathway in the geological record. Isotopic data from organic carbon in the oldest metasediments deposited ~ 3.8 Ga ago is consistent with the existence of autotrophic CO_2 fixation into biomass (Schidlowski et al., 1979; Schidlowski, 1988; Rosing, 1999). However, the specific metabolic pathway employed for carbon fixation, as well as the organisms involved, is unclear because of the absence of sedimentary carbonate from which to determine the full magnitude of isotopic fractionation (Buick, 2001). For example, the Rubisco pathway for carbon fixation used by cyanobacteria yields ^{13}C-depleted organic matter, but the pathway is also used by chemoautotrophic organisms. In addition, other carbon fixation pathways like the Acetyl Co-A pathway or the reverse TCA cycle can produce fractionations similar to that imparted by Rubisco (e.g., Fuchs, 1989). But microbial sulfate reduction is a specific pathway modulated by a unique set of enzymes across the whole biosphere and so from its existence ~ 3.5 Ga ago we have a clear picture of how complex cellular biochemistry was a billion years after the Earth formed.

10. Conclusions

Geological and biogeochemical data from the ~ 3.47-Ga North Pole barite deposit demonstrate that microscopic pyrites aligned along barite crystals were formed biologically by dissimilatory sulfate reduction, the same phenomenon first described by Hoppe–Seyler over a century ago. The existence of gypsum provided favorable conditions for mesophilic sulfate-reducing microbes producing H_2S, which was subsequently deposited as microcrystalline pyrite lining the original gypsum growth faces. This was preserved, along with a diagnostic isotopic signature, after the gypsum was hydrothermally altered to barite. This unusual sulfate-rich microenvironment of Archean age thus provides the best evidence of early metabolic processes and allows reconstruction of the primordial biogeochemical cycle for sulfur. It also permits time calibration of a deep node on the Tree of Life, and so delivers an independent way to trace the earliest evolution of life.

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